(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 406 856 B1**

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **02.03.94** (51) Int. Cl.[5]: **A61K 37/36**, A61K 47/38

(21) Application number: **90112850.4**

(22) Date of filing: **05.07.90**

(54) **Stabilized FGF composition and production thereof.**

(30) Priority: **07.07.89 JP 176228/89**
24.05.90 JP 136333/90

(43) Date of publication of application:
09.01.91 Bulletin 91/02

(45) Publication of the grant of the patent:
02.03.94 Bulletin 94/09

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(56) References cited:
EP-A- 0 308 238
EP-A- 0 312 208

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EP 0 406 856 B1

Description

The present invention relates to a stabilized fibroblast growth factor (hereinafter briefly referred to as FGF) protein composition, a method for preparing a stabilized FGF protein composition, and a method for stabilizing an FGF protein.

FGF was first isolated as a factor exhibiting strong growth promoting action on fibroblasts such as BALB/c3T3 cells [D. Gospodarowicz, Nature 249, 123 (1974)]. It is now known that the FGF exhibits growth promoting action on almost all cells derived from mesoblast. FGF is classified into basic FGF (hereinafter briefly referred to as bFGF) and acidic FGF (hereinafter briefly referred to as aFGF), based on the isoelectric point thereof. bFGF and aFGF both have strong growth promoting action and plasminogen activator inducing action on vascular endothelial cells. Together, these actions suggest a potential for the application thereof as a drug for promoting angiogenesis, as a therapeutic drug for traumas, and as a preventive and therapeutic drug for thrombosis, arteriosclerosis, etc.

Previously, the FGFs were purified to homogeneity from organs derived from animals, such as bovine pituitary. However, supply of these FGFs was limited, and there was a fear of antigenicity due to their heterozoic origin. Recently, there has been developed a method for producing FGF in large quantities. The method involves using recombinant DNA techniques to express a cloned human FGF gene in microorganisms or in animal cells. [FEBS Letters 213, 189-194 (1987); European Patent Publication (hereinafter also referred to as EP Publication) No. 237,966)].

In other way, in order to stabilize polypeptide producing factors, an aqueous medical composition characterized by comprising water-soluble polysaccharides in enough amount for stabilizing a growth factor was provided, and it is stated that the composition is effective against declining of activities of mitogen of the polypeptide growing factor and declining of bioactivities [Japanese Unexamined Patent Publication No. 63-152324/1988 corresponding to EP Publication No. 267,015]. Generally, FGF proteins are very unstable. For example, not only they are rapidly inactivated in an aqueous solution, but also inactivated during the process of lyophilization.

Further, when the FGF proteins are administered for many hours as intravenous drip, a reduction in titer during that time is unavoidable, which causes a major problem.

The above-described aqueous medical composition comprising water-soluble polysaccharides, especially in the case of cellulose derivatives of a degree of ether substitution of at least 0.35 ether groups per anhydroglucose unit in the cellulose chain, it is difficult to form solid medical composition in powder when the main ingredient is an FGF protein. Titer of the composition is lowered during mixing and drying process.

The present inventors have discovered that the stability of FGF proteins is surprisingly increased by admixing an FGF protein with a water-insoluble hydroxypropyl cellulose.

In particlular, the present inventors have succeeded in obtaining a solid composition having an improved stability of FGF protein as compared with that of the above-described aqueous medical composition comprising FGF protein and water-soluble polysaccharides.

In accordance with the present invention, there is provided (1) a stabilized FGF protein composition which comprises an FGF protein and water-insoluble hydroxypropyl cellulose; (2) a method for preparing a stabilized FGF protein composition, which comprises admixing an FGF protein with a water-insoluble hydroxypropyl cellulose; and (3) a method for stabilizing an FGF protein, which comprises admixing an FGF protein with a water-insoluble hydroxypropyl cellulose, wherein the water-insoluble hydroxypropyl cellulose is low-substituted hydroxypropyl cellulose which contains not less than 5.0 percent by weight and not more than 16.0 percent by weight of hydroxy propoxyl groups.

The FGF proteins used in the present invention may include basic FGF (hereinafter also referred to as bFGF) and acidic FGF (hereinafter also referred to as aFGF). The FGF protein used in the present invention include those derived from mammals. The mammals include human, monkey, pig, bovine, sheep and horse.

The FGF proteins include those extracted from various organs in which the presence of FGFs is already known, such as brain and pituitary.

Further, the FGF proteins include those obtained by the recombinant DNA technique [FEBS Letters 213, 189-194 (1987); EP Publication No. 237,966].

Hereinafter, the recombinant human basic FGF may be referred to as rhbFGF.

The FGF proteins used in the present invention include a FGF mutein.

Examples of the muteins of the FGFs used in the present invention include the muteins disclosed in Biochemical and Biophysical Research Communications 151, 701-708 (1988), EP No. 281,822 A2, and Japanese Patent Application No. 1-15662/1989 which corresponds to EP Publication No. 326,907 A1; and there may be included the muteins introduced by at least one glycosylation site diclosed in EP-A-394 951.

For example, the FGF muteins used in the present invention are obtained essentially by variations of the amino acid sequences of the original peptides or proteins. Such variations include addition of amino acid(s), deletion of constituent amino acid(s) and substitution of constituent amino acid(s) by different amino acid(s). Furthermore, as the muteins FGF muteins wherein glycosylation site(s) has(have) been introduced into FGF.

Such addition of amino acid(s) includes addition of at least one amino acid.

Such deletion of constituent amino acid(s) includes deletion of at least one FGF-constituent amino acid.

Such substitution of constituent amino acid(s) by different amino acid(s) includes substitution of at least one FGF-constituent amino acid by at least one different amino acid.

"At least one amino acid" in the mutein which has at least one amino acid added to the FGF, excludes methionine derived from the initiation codon used for peptide expression and as a signal peptide.

The number of the added amino acid(s) is at least one. However, it may be any number as long as FGF characteristics are not lost. More preferable amino acids include some or all of the amino acid sequences of proteins which have homology with the FGFs and which exhibit activities similar to those of the FGFs.

As for the number of the deleted FGF-constituent amino acid(s) in the mutein which lacks at least one FGF-constituent amino acid, it may be any number as long as FGF characteristics are not lost.

Examples of the deleted constituent amino acid include the 10 residues on the amino termial side of the human bFGF:

Met-Pro-Ala-Leu-Pro-Glu-Asp-Gly-Gly-Ser,

the 14 residues on the amino terminal side of the human bFGF:

```
1
Met-Pro-Ala-Leu-Pro-Glu-Asp-Gly-Gly-Ser-Gly-Ala-

     14
Phe-Pro,
```

the 41 residues on the amino terminal side of the human bFGF:

```
 1    2   3   4                    41
Met-Pro-Ala-Leu- ......        -Val,
```

the 61 residues on the carboxyl terminal side of the human bFGF:

```
 87  88                          146 147
Lys-Cys-                       -Val-Ser.
```

The muteins further include muteins lacking the 7 to 46 amino acid residues on the carboxyl side of the original peptide or protein of the bFGF.

Preferred examples of such deletion include deletion of the following amino acid sequences of the rhbFGF:

Amino acid sequence from amino acid No. 102 on
Amino acid sequence from amino acid No. 105 on
Amino acid sequence from amino acid No. 115 on
Amino acid sequence from amino acid No. 119 on
Amino acid sequence from amino acid No. 124 on
Amino acid sequence from amino acid No. 130 on
Amino acid sequence from amino acid No. 138 on

As for the number of FGF-constituent amino acids prior to substitution in the mutein, which has at least one FGF-constituent amino acid substituted by at least one different amino acid, it may be any number as long as FGF characteristics are not lost.

Examples of the constituent amino acids prior to substitution include cysteine and cystine, but cysteine is preferable. The constituent amino acids other than cysteine prior to substitution include aspartic acid, arginine, glycine and valine.

When the constituent amino acid prior to substitution is cysteine, neutral amino acids are preferable as the substituted amino acids. The neutral amino acids include glycine, valine, alanine, leucine, isoleucine, tyrosine, phenylalanine, histidine, tryptophan, serine, threonine and methionine. Serine and threonine are particularly preferred.

When the constituent amino acid prior to substitution is any one other than cysteine, amino acids which are different, for example, in hydrophilicity, hydrophobicity or electric charge from the amino acid prior to substitution are selected as the substituting different amino acids. Specifically, when the amino acid prior to substitution is aspartic acid, the substituting amino acids include asparagine, threonine, valine, phenylalanine and arginine. In particular, asparagine and arginine are preferable.

When the amino acid prior to substitution is arginine, the substituting amino acids includes glutamine, threonine, leucine, phenylalanine and aspartic acid. Glutamine is especially preferable.

When the amino acid prior to substitution is glycine, the substituting amino acids include threonine, leucine, phenylalanine, serine, glutamic acid and arginine. Threonine is particularly preferred.

When the amino acid prior to substitution is serine, the substituting amino acids include methionine, alanine, leucine, cysteine, glutamine, arginine and aspartic acid. In particular, methionine is preferable.

When the amino acid prior to substitution is valine, the substituting amino acids include serine, leucine, proline, glycine, lysine and aspartic acid. Serine is especially preferred.

As the original constituent amino acids prior to substitution, aspartic acid, arginine, glycine, serine and valine are preferably selected.

As the substituting amino acids, asparagine, glutamine, arginine, threonine, methionine, serine and leucine are preferably selected.

The most preferred substituted muteins include a mutein in which cysteine, the constituent amino acid, is substituted by serine.

In the above substitution, the substitution of at least two constituent amino acids may be simultaneously carried out. In particular, it is preferable to substitute two or three constituent amino acids.

The muteins may be obtained by a combination of two or three of the above-mentioned addition, deletion and substitution.

A mutein is preferable in which at least one human bFGF-constituent amino acid is substituted by at least one different amino acid. In particular, rhbFGF mutein CS23 is preferable in which cysteine residues at the 70- and 88-positions of human bFGF are substituted by serine residues, respectively.

The FGF mutein has had introduced at least one glycosylation site. And the mutein may further have sugar chain(s).

The glycosylation sites include a site in which an amino acid sequence constituting the glycosylation site is represented by the following the formula:

Asn-X-Y

(wherein X may be any amino acid residue, and Y is Thr, Ser or Cys).

X is preferably an amino acid other than Pro, and more preferably Gly, Tyr, Arg, Ser, Lys, Val or Ala and more preferably Gly, Lys, Val or Ala. Y is preferably Thr or Ser.

The sugar which is added to a FGF mutein may be any one found in known glycosylated proteins. Examples of such sugars include N-acetyl glycosamine, N-acetyl galactosamine, mannose, galactose, fucose and cyalic acid.

The number of sugars in a glycosyl chain is preferably at least one, and more preferably 10 to 20.

In order to produce the muteins, site-directed mutagenesis is employed. This technique is well-known and described in R. F. Lather and J. P. Lecoq, Genetic Engineering, pp. 31-50, Academic Press (1983). Mutagenesis directed to oligonucleotide is described in M. Smith and S. Gillam, Genetic Engineering: Principles and Methods, Vol. 3, pp. 1-32, Plenum Press (1981).

The production of a structural gene which encodes the mutein is carried out, for example, by the steps of:

(a) hybridizing a single-stranded DNA comprising a single strand of the structural gene of FGF with a mutagenic oligonucleotide primer(the above-mentioned primer is complementary to a region, including a codon for cysteine, to be replaced by this single strand, or including an anti-sense triplet which forms a pair with this codon in some cases, provided this does not apply to disparity with other codon for the amino acid than the above codon, or with the anti-sense triplet in some cases.),

(b) elongating the primer using DNA polymerase to form a mutational heteroduplex, and

(c) replicating this mutational heteroduplex.

Then, phage DNA for transferring the mutagenized gene is isolated and introduced into a plasmid.

A suitable host is transformed with the plasmid thus obtained, and the obtained transformant is cultivated in a medium, thereby being capable of producing the mutein.

The water-insoluble hydroxypropyl cellulose is low-substituted hydroxypropyl cellulose which is a low-substituted hydroxypropyl ether of cellulose containing not less than 5.0 percent by weight and not more than 16.0 percent by weight of hydroxypropyl groups, when dried at 105° for 1 hour (Refer to the Pharmacopeial Forum, 15, 5180 to 5181 (1989); and the Japanese Pharmacopeia, the 11th revision, 1330-1333.)

Examples of low-substituted hydroxypropyl cellulose used in the present invention include low-substituted hydroxypropyl cellulose (LH-11, LH-20, LH-21, LH-22 and LH-31, (Shin-Etsu Chemical, Japan)).

In the present invention, the weight ratio of the FGF protein to water-insoluble hydroxypropyl cellulose is preferably 1 : 0.01 to 1,000,000, more preferably 1 : 1 to 100,000, still more preferably 1 : 500 to 20,000, and especially preferably 1 : 500 to 10,000.

Further, the composition of the present invention may further contain one or more members selected from sugars, proteins, amino acids, sodium chloride and gum arabic.

The sugars include, for example, sucrose, trehalose, maltose, fructose, inositole and amylose. The proteins include, for example, casein, albumine, gelatin and egg white. The amino acids include, for example, cysteine, phenylalanine, leucine and glycine.

In the present invention, the weight ratio of FGF protein to sugars, proteins, amino acids, sodium chloride and/or gum arabic is preferably 1:0.01 to 1,000,000, more preferably 1:1 to 100,000, still more preferably 1:500 to 20,000, and especially preferably 1:500 to 10,000.

The compositions of the present invention are obtained by admixing the FGF protean with the water-insoloble hydroxypropyl cellulose, for example, by adding an aqueous solution of the FGF protein to water-insoluble hydroxypropyl cellulose in powder, followed by mixing. The pH of the aqueous solution of the FGF protein is preferably adjusted to 3 to 10, more preferably to 5 to 9.

The addition and the mixing are carried out, for example, at 10 to 30°C, preferably at 10 to 20°C.

The mixing is sufficiently performed by devices generally used for stirring and granulation [such as a mortar, a Pony mixer (Hosokawa Tekkosho, Japan), a Vertical granulator (Fuji Sangyo) and a Super mixer (Hosokawa Tekkosho)], by devices used for fluidized granulation [such as Glad (Okawara Seisakusho)) and by devices used for rolling granulation [such as CF (Freund)].

The compositions thus mixed are, for example, dried or lyophilized at room temperature (10 to 30°C) under reduced pressure (1.32 kPa (10 mmHg) or less), whereby the solid compositions stabilized in bioactivity can be obtained.

Sugars, proteins, amino acids, sodium chloride and/or gum arabic may be simultaneously added when water-insoluble hydroxypropyl cellulose and FGF protein are mixed, or they may be mixed with water-insoluble hydroxypropyl cellulose, followed by adding FGF protein. The production method of the composition is carried out by similar method with that of the composition comprising water-insoluble hydroxypropyl cellulose and FGF protein.

In the above mixing, the aqueous solution of the FGF protein stabilized with glucan sulfate may be used.

Examples of the glucan sulfate include dextran sulfates, cyclodextrin sulfates and β-1,3-glucan sulfates. All of these are sulfuric ester derivative of polymer of D-glucose. The sulfur content in the glucan sulfate is preferably not less than 3% by weight, more preferably 12 to 20% by weight, most preferably 16 to 20% by weight. In particular, dextran sulfate is preferable.

The dextran sulfates include a sulfate of dextran produced from sucrose by the action of a microorganism such as Leuconostoc mesenteroides. The dextran sulfate is a partial sulfates of dextran mainly containing $\alpha(1\rightarrow6)$ linkage, and the sulfur content therein is usually at least 12% by weight, preferably 16 to 20% by weight. The average molecular weight thereof is in the range of 1,000 to 40,000,000, preferably in the range of 3,000 to 500,000. The dextran sulfate is very easily soluble in water, and is a compound already known in the art, which is manufactured by known methods per se.

Cyclodextrin in the cyclodextrin sulfate includes cyclodextrin produced from starch by the action of a microorganism such as Bacillus macerans. The cyclodextrin has ring structure of D-glucose molecules linked by $\alpha(1\rightarrow4)$ linkage, and includes an α-type (6 molecules), a β-type (7 molecules) and a γ-type (8 molecules). In this invention, any of these forms may be used.

The cyclodextrin sulfate is obtained by sulfation of the cyclodextrin, and the sulfation is conducted according to methods already known in the art. The methods for sulfation include the methods described in U.S. Patent No. 2,923,704 and Japanese Patent Unexamined Publication No. 50-36422/1975.

The sulfur content in the cyclodextrin sulfate is usually at least 3% by weight, preferably 12 to 24% by weight. The cyclodextrin sulfate has the property of being very soluble in water.

The degree of sulfation of the cyclodextrin sulfate may be any degree as long as the sulfur content is at least 12% by weight. In particular, the cyclodextrin sulfate whose sulfur content is 16 to 21% by weight are advantageously used. Mixtures of the sulfates different from one another in degree of sulfation may be used as such, or the purified sulfates having the single degree of sulfation may be used. The purification can be conducted, for example, by concentrating a reaction solution containing an alkali metal salt of β-cyclodextrin sulfate, evaporating it to dryness, dissolving the condensate in water, and mixing the resulting aqueous solution with a hydrophilic solvent to separate a desired product.

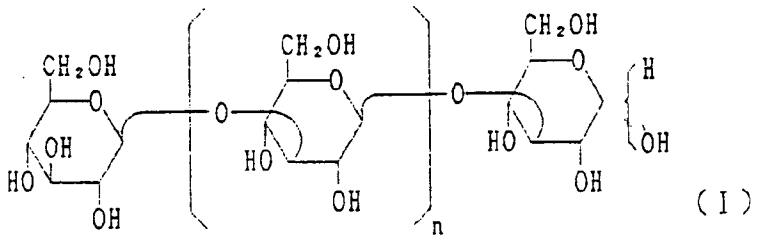
β-1,3-glucan in the β-1,3-glucan sulfate includes straight-chain β-1,3-glucans, which are produced by microorganisms belonging to Alcaligenes or Agrobacterium. There may be in the form of a low molecular weight polymer obtained by hydrolysis of the straight-chain β-1,3-glucans and similarly having a straight-chain β-1,3-glucan structure.

Curdlan (also known as a thermogelable polysaccharide PS and available from Wako Pure Chemical Industries Ltd. Japan) is known as a water-insoluble, thermogelable, unbranched glucan, and has straight-chain β-1, 3-glucan linkage alone which is produced from a microbial strain belonging to Alcaligenes or Agrobacterium [Japanese Patent Publication Nos. 43- 7000/1968, 48-32673/1973 and 48-32674/1976].

The Alcaligenes faecalis var. Myxogenes NTK-u strain, the Agrobacterium radiobacter strain and the Agrobacterium radiobacter U-19 strain, which produce Curdlan, are cited in American Type Culture Collection Catalogue of Strains, the 15th edition (1982), as ATCC- 21680, ATCC-6466 and ATCC-21679, respectively.

The properties of partially hydrolyzate of Curdlan and the method for preparation thereof have already been described in detail in Japanese Patent Unexamined Publication No. 55- 83798/1980.

Thus, the straight-chain β-1,3-glucan is a compound represented by the following formula:



(I)

wherein n is an integer of 4 to 1,000.

Any of the β-1,3-glucans described above may be used as long as the average degree of polymerization ($\overline{DP}$) thereof is not more than 1,000. In particular, there are advantageously used the partically hydrolyzed products thereof having an average degree of polymerization ($\overline{DP}$) of 6 to 300, more preferably 15 to 200.

n in the formula (I) has a relation to DP represented by the following equation:

$$\overline{DP} - 2 = n.$$

The sulfate of the straight-chain β-1,3-glucan is produced by sulfonation of the three hydroxyl groups of the intermediate monosaccharide unit of the β-1,3-glucan or its lower polymers and the hydroxyl groups of the monosaccharide units at both ends thereof. The sulfates having an average degree of substitution ($\overline{DS}$) of 0.5 to 3 per monosaccharide unit are usually used, and preferably ones having an average degree of substitution ($\overline{DS}$) of 1 to 2 are advantageously used.

Sulfation of straight-chain β-1,3-glucans or its low molecular weight polymer can be achieved by allowing a sulfating agent such as chlorosulfonic acid or sulfuric anhydride to act thereon, or by reacting a complex of sulfuric anhydride and an organic base such as pyridine, dimethylformamide, trimethylamine or dimethylaniline therewith [J. Biol. Chem. 239, 2986 (1964)].

The β-1,3-glucan sulfate is very soluble in water and low in toxicity. The sulfur content in β-1,3-glucan sulfates is usually at least 5% by weight, preferably 10 to 24% by weight.

The glucan sulfate is very low in toxicity to warm-blooded animals. This is therefore advantageous for parenteral or oral administration of the stabilized compositions comprising the FGF protein and the glucan sulfate to the warm-blooded animals.

The glucan sulfate may be used in the state of free or salt. Examples of such salts include sodium salts, potassium salts, ammonium salts and trimethylammonium salts.

When the glucan sulfate is brought into contact with the FGF protein in aqueous media, the free glucan sulfate may be added thereto, followed by addition of proper amount of an alkali or acid to give the desired pH. By the addition of alkali, the glucan sulfate may be exist in the aqueous media in the form of either its salt or a mixture of the free dextran sulfate and its salt.

If the FGF protein is brought into contact with glucan sulfate in aqueous media in the presence of an additional dibasic or tribasic carboxylic acid, the FGF protein is advantageously more stabilized.

Examples of the dibasic carboxylic acids include tartaric acid, maleic acid, malic acid and fumaric acid. The tribasic carboxylic acids include, for example, citric acid and isocitric acid.

The above carboxylic acids may be used in the form of either free compounds or their salts. Examples of such salts include sodium salts, potassium salts and ammonium salts.

Further, the free carboxylic acid may be added thereto, followed by addition of proper amounts of an alkali or acid to give the desired pH. By the addition of the alkali, the carboxylic acid may be exist in the aqueous media in the form of either its salt or a mixture of the free acid and its salt.

When the FGF protein is brought into contact with the glucan sulfate in aqueous media, it is preferred that the glucan sulfate is added in an amount of 0.1 to 100 mol/mol, more preferably 0.5 to 4 mol relative to 1 mol of FGF protein.

The concentration of the glucan sulfate in the aqueous media is preferably 0.0005 to 5% by w/v, more preferably 0.01 to 1% by w/v.

The concentration of the FGF protein in the aqueous media is preferably 0.0005 to 5% by w/v, more preferably 0.01 to 1% by w/v.

The concentration of the carboxylic acid in the aqueous media is preferably 1 mM to 1M, more preferably 10 mM to 500 mM.

For their contact in the aqueous medium, the object can be attained only by mixing the FGF protein, the glucan sulfate and the carboxylic acid as required with one another in the aqueous medium.

The aqueous media may be any media such as distilled water, physiological saline solution and glucose solution are preferably used. As the aqueous media, there can also be used buffers such as phosphate buffer and tris(hydroxymethyl)aminomethane-HCl buffer.

When the FGF protein, the glucan sulfate and the carboxylic acid as required are mixed with one another, they may be mixed as aqueous solutions, respectively, or may be mixed as solids, respectively, followed by dissolution in the aqueous medium. In mixing, the temperature is preferably 0 to 40 °C, and the pH is preferably in the range of 3 to 10, more preferably in the range of 5 to 9. The time taken to mix is usually 1 to 30 minutes.

Thus, the aqueous solution of the FGF protein stabilized with glucan sulfate is obtained.

In the present invention, the above-described composition comprising FGF protein and water-insoluble hydroxypropyl cellulose may be further coated by an enteric polymer.

Examples of the enteric polymers used in the present invention include hydroxypropyl methyl cellulose phthalate, carboxymethyl ethyl cellulose, cellulose acetate phthalate, hydroxymethyl cellulose acetate succinate and acrylic polymers [such as methacrylic acid-ethyl acrylate copolymers (Eudragit® L30D-55 and Eudragit® L100-55), methacrylic acid-methyl acrylate copolymers (Eudragit® L-100) and methacrylic acid-methyl methacrylate copolymers (Eudragit® S100), Rohm, West Germany].

The coating is conducted by known methods per se. Namely, dispersions or solutions obtained by dispersing or dissolving the coating bases in water or organic solvents are sprayed on the tablets, the granules or the fine grains by pan coating methods, fluidized coating methods, the rolling coating methods or the like. When the compositions are coated with the coating agents, it is desirable that the temperature of the composition to be coated is 25 to 70 °C, preferably 25 to 50 °C. The coating amounts are 20 to 300%, preferably 50 to 100% as the intestinally soluble polymers based on the compositions.

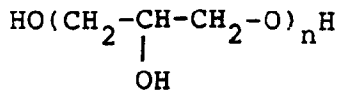
Further, the solid composition (powder) of the present invention and fatty acid ester of polyglycerol granules can also be heated and fluidized to obtain granules. According to the granules, the effective ingredient (FGF protein) of the solid composition of the present invention is stably eluted and released, and stabilized for a long time.

When the fatty acid ester of polyglycerol is a mixture, it does not show a clear melting point and is softened at a specific temperature in some cases. In this specification, the "melting point" includes a softening point which such a mixture shows.

The fatty acid ester of polyglycerol used above may be any of a monoester, a diester and a polyester as long as it is an ester formed by the combination of a polyglycerol with a fatty acid. The fatty acid ester of polyglycerol , unlike hardened oil and so on, has the characteristics of showing no crystal polymorphism

and having little interaction with effective ingredients such as drugs.

The polyglycerin is "a polyhydric alcohol having n (in a cyclic polyglycerin) to n + 2 (in a straight or branched polyglycerin) hydroxyl groups and n - 1 (in a straight or branched polyglycerin) to n (in a cyclic polyglycerin) ether combinations in one molecule" [Polyglycerin Ester, p. 12, edited and published by Sakamoto Yakuhin Kogyo Co. Ltd., Japan (May 2, 1986)]. For example, compounds represented by the following formula can be used.



wherein n indicates a degree of polymerization and is an integer of 2 or more. n is normally 2 to 50, preferably 2 to 20, more preferably 2 to 10. The polyglycerol is straight or branched.

Specific examples of such polyglycerols include diglycerol, triglycerol, tetraglycerol, pentaglycerol, hexaglycerol, heptaglycerol, octaglycerol, nonaglycerol, decaglycerol, pentadecaglycerol, eicosaglycerol and triacontaglycerol. Of these polyglycerols, for example, tetraglycerol, hexaglycerol and decaglycerol are frequently used.

The fatty acids include, for example, saturated or unsaturated higher fatty acids having 8 to 40 carbon atoms, preferably 12 to 22 carbon atoms. Examples of such fatty acids include palmitic acid, stearic acid, oleic acid, linolic acid, linolenic acid, myristic acid, lauric acid, ricinolic acid, caprylic acid, capric acid and behenic acid. Of these fatty acids, for example, stearic acid, oleic acid, lauric acid and ricinolic acid are preferable.

Specific examples of the fatty acid ester of polyglycerol include caprylyl mono(deca)glyceride, caprylyl di(tri)glyceride, lauryl mono(tetra)glyceride, lauryl mono(hexa)glyceride, lauryl mono(deca)glyceride, oleyl mono(tetra)glyceride, oleyl mono(hexa)-glyceride, oleyl mono(deca)glyceride, oleyl di(tri)glyceride, oleyl di-(tetra)glyceride, oleyl sesqui(deca)glyceride, oleyl penta(tetra)glyceride, oleyl penta(hexa)glyceride, oleyl deca(deca)glyceride, linolyl mono(hepta)glyceride, linolyl di(tri)glyceride, linolyl di(tetra)glyceride, linolyl di-(hexa)glyceride, stearyl mono(tetra)glyceride, stearyl mono(hexa)glyceride, stearyl mono(deca)glyceride, stearyl tri(tetra)glyceride, stearyl tri(hexa)glyceride, stearyl sesqui(hexa)glyceride, stearyl penta(tetra)-glyceride, stearyl penta(hexa)glycedride, stearyl deca(deca)glyceride, palmityl mono(tetra)glyceride, palmityl mono(hexa)glyceride, palmityl mono(deca)glyceride, palmityl tri(tetra)glyceride, palmityl tri(hexa)glyceride, palmityl sesqui(hexa)glycoride, palmityl penta(tetra)glyccride, palmityl penta(hexa)glyceride and palmityl deca(deca)glyceride. Examples of the preferred fatty acid ester of polyglycerol include stearyl penta(tetra)-glyceride (for example, PS-310, Sakamoto Yakuhin Co., Japan), stearyl mono(tetra)glyceride (for example, MS-310, Sakamoto Yakuhin Co.), stearyl penta(hexa)glyceride (for example, PS-500, Sakamoto Yakuhin Co.), stearyl acid sesqui(hexa)glyceride (for example, SS-500, Sakamoto Yakuhin Co.) and stearyl mono-(deca)glyceride.

These fatty acid ester of polyglycerol are used alone or as mixtures of two or more kinds.

The melting point of the fatty acid ester of polyglycerol is 40 to 80 °C, preferably 40 to 60 °C.

The molecular weight of the fatty acid ester of polyglycerol is usually 200 to 5,000, preferably 300 to 2,000. The hydrophile-lypophile balance (HLB) thereof is 1 to 22, preferably 1 to 15, and the elution rate of the effective ingredient of the powder can be controlled by adjusting the HLB. The HLB can also be adjusted by mixing two or more kinds of fatty acid ester of polyglycerol.

The fatty acid ester of polyglycerol can also be used together with lipids. As the lipids are used water-insoluble materials permissible depending on the purpose of preparations and the like. The preferred softening point or melting point of the lipids is 40 to 120 °C, particularly 40 to 90 °C.

Specific examples of the lipids include the hardened products of fats and oils such as castor oil, cotton seed oil, soybean oil, rapeseed oil and beef tallow; waxes such as beeswax, carnauba wax, spermaceti, lecitin, paraffin and microcrystalline wax; fatty acids such as stearic acid and palmitic acid, or fatty acid salts such as sodium salts and potassium salts of fatty acids; fatty alcohols such as stearyl alcohol and cetyl alcohol; and glycerides. Of these lipids, there are preferable, for example, hardened cotton seed oil, hardened castor oil, hardened soybean oil, carnauba wax, microcrystalline wax, stearic acid and stearyl alcohol.

The ratio of the lipid to the fatty acid ester of polyglycerol is usually 100 parts by weight or less of lipid per 100 parts by weight of fatty acid ester of polyglycerol, and can be suitably selected within the above range.

In the preparation of the granulated compositions, spherical fatty acid ester of polyglycerol granules are preferably used to adhere the powders (the solid compositions of the present invention) in large amounts to the fatty acid ester of polyglycerol or to allow the esters to contain the powders in large amounts, and to obtain the granulated compositions corresponding to the shape and the size of the fatty acid ester of polyglycerol granules. When the spherical fatty acid ester of polyglycerol granules are used, large amounts of powders (the solid compositions of the present invention), for example, the powder constituting 80% by weight of the whole granulated composition, can be incorporated therein. Moreover, the spherical granulated compositions relatively smooth in surface and narrow in size distribution can be obtained. In some cases, the powder can be incorporated therein so as to constitute more than 80% by weight, for example, 85% by weight, of the whole granulated composition.

The spherical fatty acid ester of polyglycerol granules can be obtained, for example, by spray cooling, preferably spray chilling. The spray chilling can be carried out by rotating a disk such as an aluminum disk having a smooth surface and dripping the molten fatty acid ester of polyglycerol thereon. The rotary disk is not particularly limited in size, but is 5 to 100 cm, preferably 10 to 20 cm in diameter. The rotational speed of the rotary disk and the dripping rate of the molten fatty acid ester of polyglycerol can be determined depending on the desired size and the like of the granules. The rotational number of the rotary disk is usually 10 to 6,000 rpm, preferably 900 to 6,000 rpm, more preferably 1,000 to 3,000 rpm. The molten fatty acid ester of polyglycerol can be dripped at a constant rate, for example, of 2 to 200 g/min, preferably 5 to 100 g/min.

The size of the fatty acid ester of polyglycerol granules can be selected according to the desired size of the granulated composition and is not particularly limited, but is usually 0.1 to 2.0 mm (10 to 150 meshes), preferably 0.15 to 0.71 mm (25 to 100 meshes).

The solid compositions (powders) of the present invention can be used together with powdery diluents. Examples of such diluents include excipients such as lactose, cornstarch, Avicel® (microcrystalline cellulose: Asahi Chemical Industry, Japan), powder sugar and magnesium stearate; binders such as starch, gelatin, gum arabic powder, methyl cellulose, carboxymethyl cellulose sodium, hydroxypropyl methyl cellulose and polyvinyl pyrrolidon; disintegrators such as carboxymethyl cellulose calcium and low substituted-hydroxypropyl cellulose; coloring agents; flavoring agents; absorbents; preservatives; wetting agents; antistatic agents; and disintegration delaying agents.

The ratio of the powder (the solid composition of the present invention) to the above fatty acid ester of polyglycerol can be established depending on the desired size of the granulated composition, the content of the drug active ingredient and the like, but is usually 10 to 1,000 parts by weight, preferably 50 to 500 parts by weight of the powder per 100 parts by weight of the fatty acid ester of polyglycerol.

The granulation by heating and fluidizing can be conducted according to conventional fluidized-bed granulating methods. The heating temperature in the granulating methods is near the melting point of the above fatty acid ester of polyglycerol ester, preferably within the range from the melting point of the fatty acid ester of polyglycerol to the temperature 5 °C lower than the melting point. If the heating temperature is too high, the fatty acid ester of polyglycerol granules tend to coalesce by fusion to form a granulated composition wide in size distribution. On the other hand, if the heating temperature is too low, it is difficult to granulate the powder (the solid composition of the present invention) with the fatty acid ester of polyglycerol granules.

The granulation can be performed by floating the fatty acid ester of polyglycerol granules and the powder (the solid composition of the present invention) to form a fluidized bed, and by heating and fluidizing them at a required temperature. It can be confirmed by the presence or absence of the powder particles whether or not the granulation is completed.

The granulated compositions thus obtained are usually fine-grained or granular.

When the granulated composition is observed under a microscope, the shape of the granule composition is similar to the original core, and it seems that the powder (solid composition of the present invention) is at least partially embedded in the fatty acid ester of polyglycerol granule, preferably involved therein to coaleace.

The compositions of the present invention thus obtained are solid.

The compositions of the present invention include pharmaceutical compositions containing the above solid compositions (for example, ointments and suppositories). Namely, in the case of the ointments, the solid compositions of the present invention are dispersed in bases for the ointments. In the case of the suppositories, the solid compositions of the present invention are dispersed in bases for the suppositories.

As the present FGF composition is stabilized, it can be advantageously used as a medicine.

The stabilized FGF protein compositions of the present invention can be safely administered parenterally or orally to warm-blooded animals (such as human, mouse, rat, hamster, rabbit, dog and cat) as such

or with pharmacologically permissible additives (such as carriers, excipients and diluents), as pharmaceutical compositions (such as tablets, capsules, granules, fine grains, powders, ointments and suppositories).

Such preparations can be formulated into the forms suitable for oral administration such as tablets, capsules, powders, granules and fine grains in accordance with known methods per se. In these cases, as additives are used excipients (such as lactose, cornstarch, light silica and fine crystalline cellulose), binders (such as alpha starch, methyl cellulose, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl celulose and polyvinyl pyrrolidone), disintegrators (such as carboxymethyl cellulose calcium, starch and low-substituted hydroxypropyl cellulose), surface active agents [such as Tween® 80 (Kao Atlas, Japan), Pluronic® F68 (Asahi Denka Kogyo, Japan) and polyoxyethylene-polyoxypropylene copolymer], antioxidants (such as L-cysteine, podium sulfite and sodium ascorbate) and lubricants (such as magnesium stearate and talc).

As to the tablets, the granules and the fine grains, coating may be carried out in accordance with known methods per se for the purpose of masking tastes or giving intragastric solubility, intestinal solubility or increasing sustained release. As the coating agents are used, for example, hydroxypropyl methyl cellulose, ethyl cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, polyoxyethylene glycol, Tween® 80, Pluronic® F68, castor oil, cellulose acetate phthalate, hydroxypropyl methyl cellulose phthalate, hydroxypropyl methyl cellulose acetate succinate, acrylic polymers (Eudragit® L100-55 and L-100, Rohm, West Germany), carboxymethyl ethyl cellulose, polyvinyl acetal diethylamino acetate, waxes and pigments such as talc, titanium oxide and red ferric oxide. These coating agents may be applied in one or more layers, alone or in combination of two or more agents.

The coating is conducted by known methods per se. Namely, dispersions or solutions obtained by dispersing or dissolving the coating bases in water or organic solvents are sprayed on the tablets, the granules or the fine grains by pan coating methods, fluidized coating methods or rolling coating methods. The tablets, the granules and the fine grains are preferably coated at 25 to 70 °C, more preferably at 25 to 50 °C.

Further, ointments and suppositories can be prepared according to known methods per se using the following additives.

Examples of the additives used when the ointments are prepared include vaseline, beweswax, paraffin, liquid paraffin, cholesterol, stearyl alcohol, lanolin, cetyl alcohol and polyethylene glycol.

Examples of the additives used when the suppositories are prepared include cacao butter, hydrogenated vegetable oils, monoglycerides, triglycerides, glycerogelatin and polyethylene glycol.

The FGF protein compositions of the present invention have growth promoting action on fibroblasts, high stability and low toxicity. Therefore, the FGF protein compositions can be used as therapeutic promoting drugs for burns, traumas, postoperative tissues and the like, or therapeutic drugs for thrombosis, arteriosclerosis and the like by arterializing action. Also, they can be used as reagents for promoting cell cultivation.

When the FGF protein compositions of the present invention are used as the above-mentioned drugs, they are administered, for example, to the above-mentioned warm-blooded animals in an appropriate amount ranging from about 1 ng/kg to 100 μg/kg daily as the FGF protein, taking into account the route of administration, symptoms, etc.

Recombinant human bFGF mutein CS23 (hereinafter also referred to as rhbFGF mutein CS23) used in Examples hereinafter described is prepared by the method described in Biochemical and Biophysical Research Communications 151, 701-708 (1988) or the method described in European Patent Publication No. 281,822 A2. The rhbFGF mutein CS23 used in Examples hereinafter described was prepared and purified by the methods described in European Patent Publication No. 281,822 A2, Examples 1, 2, 7 and 24, using transformant Escherichia coli MM294/pTB762 (IFO 14613, FERM BP-1645).

The transformant Escherichia coli MM294/pTB762 described above was deposited with the Institute for Fermentation, Osaka (IFO), Japan and with the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry (FRI), Japan. The accession number and the deposit date are shown in Table 1. As to the deposit in FRI, the deposit was initially made under accession number denoted by FERM P number. Said deposit was converted to the deposit under Budapest Treaty and the transformant has been stored at FRI under accession number denoted by FERM BP.

Table 1

Transformant	IFO	FRI
E. coli MM294/pTB762	IFO 14613 (May 27,1987)	FERM P-9409 FERM BP-1645 (June 11, 1987)

Reference Example 1

The FGF activity in Examples described below was measured by the following method.

Samples diluted in 2-fold step with DMEM medium containing 10% calf serum were added to a Nunc 96-well microtiter plate (flat base) in an amount of 50 μl per well, and then each well was seeded with 50 μl (2 X 10^3 cells) of fetal bovine cardiac endotherial cells (CRL1395) purchased from American Type Culture Clollection, followed by cultivation for 3 days. Then, to each well was added 20 μl of MTT [3-(4,5-dimethyazolyl-2-yl)-2,5-diphenyltetrazolium bromide] [Journal of Immunological Method 93, 157 (1986)] solution (5 mg/ml PBS, Sigma). After 4.5 hours, 100 μl of 10% SDS-0.01 N HCl was added thereto, and then the microtiter plate was allowed to stand overnight. Thereafter, the absorbance at 590 nm was measured by using Titertek Multiscan [Tada et al., Journal of Immunological Method 93, 157 (1986)].

Example 1

Sodium dextran sulfate having a mean molecular weight of 7,500 (Seikagaku Kogyo, Japan) was added to a 50 mM sodium citrate solution (pH 8.0) containing rhbFGF mutein CS23 in a concentration of 450 μg/ml so as to give a concentration of 210 μg/ml. Then, 1 ml of the resulting solution was added to 5 g of low-substituted hydroxypropyl cellulose (hereinafter referred to as L-HPC) (LH-20, hydroxypropoxyl group : 13.0 to 16.0%, Shin-Etsu Chemical), followed by sufficient stirring. The mixture thus obtained was dried at room temperature (20 °C) under reduced pressure (0.66 kPa (5 mm Hg)) for 20 hours to obtain a crude powder composition containing rhbFGF mutein CS23 and L-HPC.

As a control, a powder composition containing rhbFGF mutein CS23 and lactose was prepared in the same manner as the above method except that 5 g of lactose was used in place of L-HPC.

The remaining activity of these compositions was measured by the method described in Reference Example 1. The results are shown in Table 2.

Table 2

Additive	Remaining FGF Activity (%)
L-HPC	127
Lactose	8

Example 2

Sodium dextran sulfate having a mean molecular weight of 7,500 was added to a 50 mM sodium citrate solution (pH 8.0) containing rhbFGF mutein CS23 in a concentration of 500 μg/ml so as to give a concentration of 233 μg/ml. Then, 1 ml portions of the resulting solution were added to 5 g of L-HPC (LH-11, hydroxypropoxyl group: 10.0 to 13.0%, Shin-Etsu Chemical) and to 5 g of lactose, respectively, followed by sufficient stirring. The mixtures thus obtained were lyophilized to obtain powder compositions containing rhbFGF mutein CS23 and L-HPC, and rhbFGF mutein CS23 and lactose, respectively.

The remaining activity of these compositions is shown in Table 3.

Table 3

Additive	Remaining FGF Activity (%)
L-HPC	85
Lactose	11

Example 3

Using the powder composition containing rhbFGF mutein CS23 and L-HPC obtained in Example 1, granules were prepared by the following method.

Namely, 85 g of nonpareils (590 to 840 μm (20 to 28 meshes)) was placed in a mini CF device (Freund), and coated with the powder having the following composition by sprinkling at a rate of 5 g/min at a rotational speed of a rotor of 400 rpm while spraying 50 ml of a 1% (w/v) hydroxypropyl cellulose (hydroxypropoxyl group : 53.4 to 77.5%) solution at a rate of 2.5 ml/min. The resulting product was dried under vacuum at 40 °C for 16 hours, followed by sieving through a round sieve to obtain spherical granules having a particle size of 500 to 1410 μm (12 to 32 meshes).

[Powder]	
Powder composition containing the rhbFGF mutein CS23 obtained in Example 1	20 g
Fine granulated sugar	20 g
Corn Starch	20 g

Then, 60 g of the granules thus obtained was placed in a mini CF device (Freund), and provided with an intestinally soluble coating by spraying the intestinally soluble film solution having the following composition at a rate of 5 ml/min at a rotational speed of a rotor of 400 rpm, adjusting the air temperature to 40 °C and the granule temperature to 35 °C, to obtain intestinally soluble granules.

[Intestinally Soluble Film Solution]	
Hydroxypropyl methyl cellulose phthalate	20 g
Castor oil	2 g
Tale	0.4g
Acetone	200 ml

Example 4

2 ml of 50 mM sodium citrate solution (pH 7.0) containing rhbFGF mutein CS23 at a concentration of 1 mg/ml was added to 2 g of L-HPC (LH-20, Shin-Etsu Chemical), and the mixture was sufficiently stirred, followed by drying at room temperature (20 °C) under reduced pressure (0.66 kPa (5 mm Hg)) for 20 hours to obtain a powder composition. The remaining activity of the resulting composition was 100%.

Example 5

2 ml of 50 mM sodium citrate solution (pH 7.0) containing rhbFGF mutein CS23 at a concentration of 1 mg/ml was added to a mixture of 1.6 g of L-HPC (LH-20, Shin-Etsu Chemical) and 0.4 g of powder sugar (pulverized sucrose) as a saccharide, and the resulting mixture was sufficiently stirred, followed by drying at room temperature (20 °C) under reduced pressure (0.66 kPa (5 mm Hg)) for 20 hours to obtain a powder composition. The composition thus obtained showed the following remaining activity immediately after preparation, after 2 months at 40 °C and after 6 months at 40 °C.

	L-HPC + Sugar Powder
Immediately after preparation	95%
After 2 months at 40 °C	81%
After 6 months at 40 °C	81%

Example 6

(1) 500 g of stearyl mono(tetra)glyceride (MS-310, Sakamoto Yakuhin Co.) was added to 500 g of stearyl penta(tetra)glyceride (PS-310, Sakamoto Yakuhin Co.), and the mixture was heated at 90 °C to melt it. The resulting melt was dripped at a rate of 20 g/minute on an aluminum disk 15 cm in diameter rotating at 1,000 rpm to prepare spherical fatty acid ester of polyglycerol granules which pass through a 500 μm (32-mesh) sieve, but does not pass through a 350 μm (42-mesh) sieve.

100 g of the spherical fatty acid ester of polyglycerol granules obtained above, 5 g of the powder composition obtained in Example 4 and 95 g of L-HPC (LH-20, Shin-Etsu Chemical) were placed in a fluidized granulator (type FD-3S, Fuji Sangyo). Setting the supply air temperature to 54 °C, the mixture was heated and fluidized. After it was confirmed that L-HPC particles floating in a fluidized bed had disappeared, the supply of heat was stopped and the cooling was carried out, thereby obtaining granules.

(2) 100 g of the granules of rhbFGF mutein CS23 obtained in the above item (1) was placed in the fluidized granulator (type FD-3S, Fuji Sangyo), and coated with a coating solution [a solution of 100 g of hydroxypropyl methyl cellulose phthalate HP-55S (Shin-Etsu Chemical) in 1 litre of a 1:1 mixture of acetone and ethanol) at a solution supply rate of 2 g/minute at a supply air temperature of 48 °C to obtain a coated granule composition containing rhbFGF mutein CS23.

Example 7

2 ml of 50 mM sodium citrate solution (pH 7.0) containing rhbFGF mutein CS23 at a concentration of 1 mg/ml was added to a mixture of 1.6 g of L-HPC (LH-20, Shin-Etsu Chemical) and 0.4 g of human serum albumin (HSA), casein or purified gelatin as a protein, and the resulting mixture was sufficiently stirred, followed by drying at room temperature (20 °C) under reduced pressure (0.66 kPa (5 mm Hg)) for 20 hours to obtain a respective powder compositions.

Example 8

2 ml of 50 mM sodium citrate solution (pH 7.0) containing rhbFGF mutein CS23 at a concentration of 1 mg/ml was added to a mixture of 1.6 g of L-HPC (LH-20, Shin-Etsu Chemical) and 0.4 g of sodium chloride, and the resulting mixture was sufficiently stirred, followed by drying at room temperature (20 °C) under reduced pressure (0.66 kPa (5 mm Hg)) for 20 hours to obtain a powder composition.

Example 9

2 ml of 50 mM sodium citrate solution (pH 7.0) containing rhbFGF mutein CS23 at a concentration of 1 mg/ml was added to a mixture of 1.6 g of L-HPC (LH-20, Shin-Etsu Chemical) and 0.4 g of L-cysteine as an amino acid, and the resulting mixture was sufficiently stirred, followed by drying at room temperature (20 °C) under reduced pressure (0.66 kPa (5 mm Hg)) for 20 hours to obtain a powder composition.

Example 10

2 ml of 50 mM sodium citrate solution (pH 7.0) containing rhbFGF mutein CS23 at a concentration of 1 mg/ml was added to a mixture of 1.2 g of L-HPC (LH-20, Shin-Etsu Chemical), 0.4 g of L-cysteine and 0.4 g of sodium chloride, and the resulting mixture was sufficiently stirred, followed by drying at room temperature (20 °C) under reduced pressure (0.66 kPa (5 mm Hg)) for 20 hours to obtain a powder composition.

Example 11

2 ml of 50 mM sodium citrate solution (pH 7.0) containing rhbFGF mutein CS23 at a concentration of 1 mg/ml was added to a mixture of 1.2 g of L-HPC (LH-20, Shin-Etsu Chemical), 0.4 g of L-cysteine and 0.4 g of powder sugar, and the resulting mixture was sufficiently stirred, followed by drying at room temperature (20 °C) under reduced pressure (0.66 kPa (5 mm Hg)) for 20 hours to obtain a powder composition.

Example 12

2 ml of 50 mM sodium citrate solution (pH 7.0) containing rhbFGF mutein CS23 at a concentration of 1 mg/ml was added to a mixture of 1.6 g of L-HPC (LH-20, Shin-Etsu Chemical) and 0.4 g of gum arabic, and the resulting mixture was sufficiently stirred, followed by drying at room temperature (20 °C) under reduced pressure (0.66 kPa (5 mm Hg)) for 20 hours to obtain a powder composition.

Experimental Example 1

Sodium dextran sulfate having an average molecular weight of 7,500 was added to a 50 mM sodium citrate solution (pH 8.0) containing rhbFGF mutein CS23 in a concentration of 200 μg/ml so as to give a concentration of 93.2 μg/ml. Then, 1 ml portions of the resulting solution were added to 1 g of L-HPC (LH-20) and to 1 g of hydroxypropyl cellulose (hydroxypropoxyl group : 61 %), respectively. The resulting solution was stirred sufficiently. The mixtures thus obtained were dried at room temperature for 20 hours under reduced pressure (20 °C, 0.66 kPa (5 mmHg)) to give powder compositions containing rhbFGF mutein CS23 and L-HPC, and rhbFGF mutein CS23 and hydroxypropyl cellulose, respectively. The remaining activity of these compositions is shown in Table 4.

Table 4

Additive	Remaining FGF Activity (%)
L-HPC	100
Hydroxypropyl cellulose	44

Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. A stabilized FGF protein composition which comprises an FGF protein and water-insoluble hydroxypropyl cellulose, wherein the water-insoluble hydroxypropyl cellulose is low-substituted hydroxypropyl cellulose which contains not less than 5.0 percent by weight and not more than 16.0 percent by weight of hydroxypropyl groups.

2. A composition in accordance with claim 1, wherein the FGF protein is an FGF mutein.

3. A composition in accordance with claim 2, wherein the FGF protein is a mutein at least one human basic FGF-constituent amino acid of which is substituted by at least one different amino acid.

4. A composition in accordance with claim 1, which is further coated by an enteric polymer.

5. A method for preparing a stabilized FGF protein composition, which comprises admixing an FGF protein with a water-insoluble hydroxypropyl cellulose as defined in claim 1.

6. A method in accordance with claim 5, which comprises further coating the composition by an enteric polymer.

7. A method for stabilizing an FGF protein, which comprises admixing an FGF protein with a water-insoluble hydroxypropyl cellulose as defined in claim 1.

8. A method in accordance with claim 7, which comprises further coating the composition by an enteric polymer.

Claims for the following Contracting States : ES, GR

1. A method for preparing a stabilized FGF protein composition, which comprises admixing an FGF protein with a water-insoluble hydroxypropyl cellulose, wherein the water-insoluble hydroxypropyl cellulose is low-substituted hydroxypropyl cellulose which contains not less than 5.0 percent by weight and not more than 16.0 percent by weight of hydroxypropoxyl groups.

2. A method in accordance with claim 1, wherein the FGF protein is an FGF mutein.

3. A method in accordance with claim 2, wherein the FGF protein is a mutein at least one human basic FGF-constituent amino acid of which is substituted by at least one different amino acid.

4. A method in accordance with claim 1, which comprises further coating the composition by an enteric polymer.

5. A method for stabilizing an FGF protein, which comprises admixing an FGF protein with a water-insoluble hydroxypropyl cellulose as defined in claim 1.

6. A method in accordance with claim 5, wherein the FGF protein is an FGF mutein.

7. A method in accordance with claim 6, wherein the FGF protein is a mutein at least one human basic FGF-constituent amino acid of which is substituted by at least one different amino acid.

8. A method in accordance with claim 5, which comprises further coating the composition by an enteric polymer.

Patentansprüche
Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Stabilisierte FGF-Proteinzusammensetzung, die ein FGF-Protein und wasserunlösliche Hydroxypropylzellulose umfaßt, worin die wasserunlösliche Hydroxypropylzellulose niedrigsubstituierte Hydroxypropylzellulose ist, die nicht Weniger als 5,0 Gew.-% und nicht mehr als 16,0 Gew.-% Hydroxypropylgruppen enthält.

2. Zusammensetzung nach Anspruch 1, worin das FGF-Protein ein FGF-Mutein ist.

3. Zusammensetzung nach Anspruch 2, worin das FGF-Protein ein Mutein ist, bei dem zumindest eine menschliche basische FGF-konstituierende Aminosäure durch zumindest eine andere Aminosäure ersetzt ist.

4. Zusammensetzung nach Anspruch 1, die weiters mit einem enterischen Polymer beschichtet ist.

5. Verfahren zur Herstellung einer stabilisierten FGF-Proteinzusammensetzung, welches das Mischen eines FGF-Proteins mit einer wasserunlöslichen Hydroxypropylzellulose wie in Anspruch 1 definiert umfaßt.

6. Verfahren nach Anspruch 5, das weiters das Beschichten der Zusammensetzung mit einem enterischen Polymer umfaßt.

7. Verfahren zum Stabilisieren eines FGF-Proteins, welches das Mischen eines FGF-Proteins mit einer wasserunlöslichen Hydroxypropylzellulose wie in Anspruch 1 definiert umfaßt.

8. Verfahren nach Anspruch 7, welches weiters das Beschichten der Zusammensetzung mit einem enterischen Polymer umfaßt.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zur Herstellung einer stabilisierten FGF-Proteinzusammensetzung, die das Vermischen eines FGF-Proteins mit einer wasserunlöslichen Hydroxypropylzellulose umfaßt, worin die wasserunlösliche Hydroxypropylzellulose niedrigsubstituierte Hydroxypropylzellulose ist, die nicht weniger als 5,0 Gew.-% und nicht mehr als 16,0 Gew.-% Hydroxypropylgruppen enthält.

2. Verfahren nach Anspruch 1, worin das FGF-Protein ein FGF-Mutein ist.

3. Verfahren nach Anspruch 2, worin das FGF-Protein ein Mutein ist, bei dem zumindest eine menschliche basische FGF-konstituierende Aminosäure durch zumindest eine andere andere Aminosäure ersetzt ist.

4. Verfahren nach Anspruch 1, welches weiters das Beschichten der Zusammensetzung mit einem enterischen Polymer umfaßt.

5. Verfahren zum Stabilisieren eines FGF-Proteins, welches das Mischen eines FGF-Proteins mit einer wasserunlöslichen Hydroxypropylzellulose wie in Anspruch 1 definiert umfaßt.

6. Verfahren nach Anspruch 5, worin das FGF-Protein ein FGF-Mutein ist.

7. Verfahren nach Anspruch 6, worin das FGF-Protein ein Mutein ist, bei dem zumindest eine menschliche basische FGF-konstituierende Aminosäure durch zumindest eine andere Aminosäure ersetzt ist.

8. Verfahren nach Anspruch 5, welches weiters das Beschichten der Zusammensetzung mit einem enterischen Polymer umfaßt.

Revendications
Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Composition de protéine FGF stabilisée, qui comprend une protéine FGF et de l'hydroxypropyl-cellulose insoluble dans l'eau, et dans laquelle l'hydroxypropyl-cellulose insoluble dans l'eau est une hydroxypropyl-cellulose à faible degré de substitution qui ne contient pas moins de 5,0 % en poids et pas plus de 16,0 % en poids de groupes hydroxypropyles.

2. Composition conforme à la revendication 1, dans laquelle la protéine FGF est une mutéine FGF.

3. Composition conforme à la revendication 2, dans laquelle la protéine FGF est une mutéine FGF dans laquelle au moins un acide aminé constitutif du FGF basique humain a été remplacé par au moins un acide aminé différent.

4. Composition conforme à la revendication 1, qui est en outre enrobée d'un polymère entérosoluble.

5. Procédé de préparation d'une composition de protéine FGF stabilisée, qui comporte le fait de mélanger une protéine FGF avec une hydroxypropyl-cellulose insoluble dans l'eau, telle qu'on l'a définie dans la revendication 1.

6. Procédé conforme à la revendication 5, qui comporte en outre le fait d'enrober la composition avec un polymère entérosoluble.

7. Procédé de stabilisation d'une protéine FGF, qui comporte le fait de mélanger une protéine FGF avec une hydroxypropyl-cellulose insoluble dans l'eau, telle qu'on l'a définie dans la revendication 1.

8. Procédé conforme à la revendication 7, qui comporte en outre le fait d'enrober la composition avec un polymère entérosoluble.

Revendications pour les Etats contractants suivants : ES, GR

1. Procédé de préparation d'une composition de protéine FGF stabilisée, qui comprend le fait de mélanger une protéine FGF avec une hydroxypropyl-cellulose insoluble dans l'eau, et dans lequel l'hydroxypropyl-cellulose insoluble dans l'eau est une hydroxypropyl-cellulose à faible degré de substitution qui ne contient pas moins de 5,0 % en poids et pas plus de 16,0 % en poids de groupes hydroxypropyles.

2. Procédé conforme à la revendication 1, dans lequel la protéine FGF est une mutéine FGF.

3. Procédé conforme à la revendication 2, dans lequel la protéine FGF est une mutéine FGF dans laquelle au moins un acide aminé constitutif du FGF basique humain a été remplacé par au moins un acide aminé différent.

4. Procédé conforme à la revendication 1, qui comporte en outre le fait d'enrober la composition avec un polymère entérosoluble.

5. Procédé de stabilisation d'une protéine FGF, qui comporte le fait de mélanger une protéine FGF avec une hydroxypropyl-cellulose insoluble dans l'eau, telle qu'on l'a définie dans la revendication 1.

6. Procédé conforme à la revendication 5, dans lequel la protéine FGF est une mutéine FGF.

7. Procédé conforme à la revendication 6, dans lequel la protéine FGF est une mutéine FGF dans laquelle au moins un acide aminé constitutif du FGF basique humain a été remplacé par au moins un acide aminé différent.

8. Procédé conforme à la revendication 5, qui comporte en outre le fait d'enrober la composition avec un polymère entérosoluble.